# Association of methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphisms (C677T and A1298C) with thyroid dysfunction: A meta-analysis and trial sequential analysis

Rui Yang<sup>1</sup>\* https://orcid.org/ 0000-0002-1954-8071

Danhua Pu<sup>1</sup>\* https://orcid.org/ 0000-0002-5995-4816

Rongrong Tan<sup>1</sup> https://orcid.org/ 0000-0001-8261-2534

Jie Wu<sup>1</sup> https://orcid.org/ 0000-0003-2321-1866

### ABSTRACT

Recent studies have shown that two common methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms (C677T and A1298C) might correlate with thyroid dysfunction, but the results remain inconsistent. We carried out a meta-analysis aiming to assess the relationship of both polymorphisms with thyroid dysfunction. The PubMed, EMBASE, CNKI (China National Knowledge Infrastructure), CBMdisc (China Biology Medicine disc), WeiPu and Wanfang databases were searched up to September 2021. Case-control and cohort studies on MTHFR polymorphism and thyroid dysfunction were identified. Eight studies from six publications were finally included in our meta-analysis, including 817 patients and 566 controls. After pooled analysis, we found that the MTHFR C677T polymorphism was associated with an increased risk of hypothyroidism (TT vs. CC+CT/recessive model: OR = 2.07, 95% CI: 1.02-4.20, P = 0.04; TT vs. CC/homozygote model: OR = 2.35, 95% CI: 1.13-4.86, P = 0.02), while trial sequential analysis (TSA) revealed that it could be a false positive result. The MTHFR A1298C polymorphism was related to a decreased risk of hypothyroidism (C vs. A/allele model: OR = 0.63, 95% Cl: 0.44-0.92, P = 0.02; CC vs. AC+AA/recessive model: OR = 0.42, 95% CI: 0.22-0.79, P = 0.007; CC vs. AA/homozygote model: OR = 0.43, 95% CI: 0.25-0.85, P = 0.02), which was conclusive according to TSA. The results of this meta-analysis suggest that MTHFR A1298C seems to be a protective factor for hypothyroidism, while the MTHFR C677T polymorphism may be a risk factor. However, more well-designed studies with larger sample sizes are needed to obtain more reliable results of the association between the MTHFR C677T polymorphism and hypothyroidism. Arch Endocrinol Metab. 2022;66(4):551-81

#### Keywords

Thyroid dysfunction; methylenetetrahydrofolate reductase (MTHFR); polymorphism; risk

<sup>1</sup> State Key Laboratory of Reproductive Medicine, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Jiangsu Women and Children Health Hospital, Nanjing, China

\*These authors contributed equally to this work. Rui Yang and Danhua Pu were the principal investigators of this study, who were responsible for data collection, analysis and the writing of the manuscript. Rongrong Tan helped in analysis and the revising of the manuscript. Jie Wu developed the hypothesis and study design and supervised this study. All authors approved the final paper for submission.

#### Correspondence to:

Jie Wu State Key Laboratory of Reproductive Medicine, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Jiangsu Women and Children Health Hospital, Nanjing, China 368 Jiangdong North Road, Jiangsu Women and Children Health Hospital, Nanjing, Jiangsu, 210036, China wujiemd@126.com

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INTRODUCTION

Thyroid dysfunction is a common endocrine disorder that always results from autoimmune thyroid diseases (AITDs), such as Hashimoto's thyroiditis (HT) and Graves' disease (GD). AITDs can affect people at any age, but women of reproductive age (30~50 years old) are more likely to suffer from these diseases (1,2). Thyroid dysfunction is associated with several adverse perinatal outcomes in the mother and fetus, including infertility, miscarriage, hypertensive disorders, premature delivery, and decreased IQ in the offspring (3,4). Recently, several clinical and epidemiological studies have shown a relationship between methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms and thyroid dysfunction (5-7). One of the publications reported that two women diagnosed with Hashimoto's thyroiditis suffered from infertility, and in both, *MTHFR* gene polymorphisms were identified (7).



Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism that catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. MTHFR plays a crucial role in the regulation of DNA synthesis, the methylation cycle, and homocysteine concentrations in the blood (5). The *MTHFR* gene has been mapped to chromosomal region 1p36.22 and consists of 12 exons, encoding the 656-amino-acid protein.

C677T (rs1801133) and A1298C (rs1801131) are the two most common single nucleotide polymorphisms (SNPs) in the MTHFR gene (8,9). A C to T substitution at the 677th nucleotide of the MTHFR gene converts an alanine to a valine and causes thermolability of MTHFR (8). The MTHFR A1298C polymorphism results in a glutamic acid-to-alanine substitution leading to lower MTHFR enzyme activity than wildtype (10). A large body of literature has reported that genetic variation in this gene is associated with many diseases, such as neural tube defects, Alzheimer's disease, vascular diseases and some kinds of cancer (11-17). Moreover, several studies have indicated that patients with hypothyroidism have elevated serum total homocysteine (18-26), which seemed to implicate the underlying correlation between the MTHFR gene polymorphism and hypothyroidism.

To date, several studies (5,6,27-32) have been carried out to explore the potential association between *MTHFR* gene polymorphisms and thyroid diseases (HT, GD, and subclinical/overt hyper- and hypothyroidism), but the results remain controversial. Here, we conducted a meta-analysis of all case-control and cohort studies to shed some light on the association between the *MTHFR* C677T and A1298C polymorphisms and thyroid dysfunction.

### MATERIALS AND METHODS

#### Search strategy

PubMed (1950-2021), EMBASE (1974-2021) and Chinese databases, including the China National Knowledge Infrastructure (CNKI), China Biology Medicine disc (CBMdisc), WeiPu and Wanfang databases, were searched up to September 2021. Two authors independently performed a comprehensive literature search for relevant studies using the following terms: ("Methylenetetrahydrofolate reductase" OR "MTHFR") AND ("thyroid" OR "thyroid diseases"

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OR "thyroid dysfunction" OR "hyperthyroidism" OR "hypothyroidism" OR "autoimmune thyroid disease" OR "Graves' disease" OR "Hashimoto's thyroiditis"). All references cited in the included studies or relevant reviews were also hand searched to identify any additional articles.

#### Inclusion and exclusion criteria

Eligible studies included in the meta-analysis met the following inclusion criteria: (1) estimated the association between the *MTHFR* C677T and/or A1298C polymorphism and thyroid dysfunction (subclinical or overt hypo- and/or hyperthyroidism) or autoimmune thyroid diseases (Graves' disease and/ or Hashimoto's thyroiditis); (2) were case-control or cohort studies; and (3) provided enough information on the frequency of genotypes in cases and controls. The exclusion criteria were as follows: (1) review articles, animal studies, simple commentaries, case reports, or unpublished reports and (2) reports containing no usable data.

### Quality evaluation and data extraction

The quality of the included studies was evaluated according to the Newcastle-Ottawa Scale (NOS), and only studies with a quality score of 6 or better were included for further analyses. Two authors independently extracted data from all eligible studies. Disagreement was settled by discussions. For each of the included studies, the following data were collected: the first author's last name, publication year, country, ethnicity, thyroid function and sample size of cases and controls. Hardy-Weinberg equilibrium (HWE) in the controls was also performed as another reference to determine the quality of eligible studies.

#### **Statistical analysis**

The associations between the *MTHFR* C677T and/ or A1298C polymorphism and thyroid diseases were assessed by calculating the pooled odds ratios (ORs) and 95% confidence intervals (95% CIs). The statistical significance of the summary OR was determined with the Z-test. Five models, including the allele model (C677T: T vs. C; A1298C: C vs. A), dominant model (C677T: TT+TC vs. CC; A1298C: CC+AC vs. AA), recessive model (C677T: TT vs. TC+CC; A1298C: CC vs. AC+AA), homozygote model (C677T: TT vs. CC; A1298C: CC vs. AA) and heterozygote model (C677T: TC vs. CC; A1298C: AC vs. AA), were compared. The heterogeneity across the studies was estimated by the chisquare-based Q statistic test and the I<sup>2</sup> test. The fixedeffect model (using the Mantel-Haenszel method) was used if  $I^2 \leq 50\%$ ; otherwise, the random-effect model (using the DerSimonian-Laird method) was applied. Subgroup analyses were performed by ethnicity and thyroid function. The Revman 5.3 (Review Manager Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used for all statistical analyses. Stata software (version 16.0; Stata Corp LP, College Station, TX, USA) was used to analyze publication bias and for sensitivity analysis. Publication bias was investigated with Begg's funnel plot, and funnel plot asymmetry was further assessed by Egger's linear regression test (33). The significance of the intercept was determined by the t-test, and a P-value less than 0.05 was considered statistically significant. Sensitivity analysis was conducted by removing each individual study sequentially from the analysis to examine the effect of a single study on the collective results.

### **Trial sequential analysis**

TSA 0.9.5.10 software (http://www.ctu.dk/tsa/) was used for trial sequential analysis to minimize the type-I error and random error (34). The required information size (RIS) was determined based on a 5% risk of type I error, an 80% power of the study, and a case-control event proportion calculated from meta-analysis by the weighted average. The O'Brien-Fleming boundary or futility boundary was constructed to determine whether the present meta-analysis was sufficiently powered and conclusive. If the Z-curve crosses the TSA boundaries or futility area, there is sufficient information to support the conclusions, and further trials are unlikely to change the findings. If the Z-curve does not cross any of the boundaries or reach the RIS, the evidence is insufficient to make a firm conclusion. TSA would be conducted in the allele model. Meta-analysis, which presented a significant result in the pooled analysis, was also tested under TSA.

### RESULTS

#### **Study selection**

Relevant citations were retrieved and preliminarily screened. Seventy studies were identified after discarding

duplicates, and fifty-eight were excluded because they were thematically irrelevant based on the title and abstract. Thus, the full text of thirteen studies was searched and assessed. One conference abstract (35) was excluded due to a lack of detailed information. A case report (7) and three (20,27,29) case-only studies were deleted. A case-control study was excluded because the data were unusable (36). Finally, a total of seven papers (5,6,28,30-32,37) were eligible for quality evaluation (Table S1), but one publication (28) was excluded for its low NOS scores. Therefore, six publications (5,6,30-32,37) were eligible for data extraction, two (6,31) of which contained two separate studies. Therefore, eight studies from six publications were ultimately included in this meta-analysis (Figure 1).

In the present meta-analysis, eight studies met our criteria for *MTHFR* C677T polymorphism metaanalysis, and seven studies were eligible for *MTHFR* A1298C polymorphism meta-analysis. All the included studies were divided into "hyperthyroidism", "hypothyroidism" or "not applicable (NA)" groups according to the thyroid function of the cases. The distributions of the *MTHFR* C677T and A1298C genotypes and the HWE of the included studies are shown in Table 1.



Figure 1. Flow chart of the study selection process.

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Chudiaa	Ocumbrus	Filminite	Turner	Thyroid		Cases		(	Controls	5	Puwe
Studies	Country	Ethnicity	Types	function	CC	CT	TT	CC	CT	TT	- P <sub>HWE</sub>
(MTHFR C677T)											
Mao <i>et al.,</i> 2010	China	Asian	Graves' disease	hyperthyroidism	51	88	60	36	131	68	0.0371
Arakawa <i>et al.,</i> 2012	Japan	Asian	Hashimoto's disease	NA	45	63	11	36	35	13	0.3636
			Graves' disease	NA	54	79	27				
			Hashimoto's disease and Gra	ives' disease	99	142	38				
Lee et al. , 2016	Korea	Asian	Graves' disease	hyperthyroidism	35	55	32	35	53	12	0.2332
Kvaratskhelia et al., 2017	Georgia	Caucasian	Subclinical hypothyroidism	hypothyroidism	8	6	5	14	4	1	0.3638
Abu-Hassan et al., 2019	Jordan	Caucasian	Hypothyroidism	hypothyroidism	49	32	17	55	32	12	0.0432
			Hyperthyroidism	hyperthyroidism	51	10	5				
			Hypothyroidism and Hyperthy	roidism	100	42	22				
Kvaratskhelia et al., 2020	Georgia	Caucasian	hypothyroidism	hypothyroidism	16	15	3	25	4	0	0.6900
				Total		817			566		
(MTHFR A1298C)					AA	AC	CC	AA	AC	CC	
Mao <i>et al.</i> , 2010	China	Asian	Graves' disease	hyperthyroidism	139	41	6	178	55	2	0.3115
Arakawa <i>et al.</i> , 2012	Japan	Asian	Hashimoto's disease	NA	75	36	3	37	26	1	0.1314
			Graves' disease	NA	95	60	2				
			Hashimoto's disease and Gra	ives' disease	170	96	5				
Lee <i>et al.</i> , 2016	Korea	Asian	Graves' disease	hyperthyroidism	88	34	0	72	26	2	0.8445
Abu-Hassan et al., 2019	Jordan	Caucasian	Hypothyroidism	hypothyroidism	45	37	16	37	29	32	0.0001
			Hyperthyroidism	hyperthyroidism	6	46	14				
			Hypothyroidism and Hyperthy	roidism	51	83	30				
Kvaratskhelia et al., 2020	Georgia	Caucasian	hypothyroidism	hypothyroidism	26	6	2	22	4	3	0.0054
				Total		777			526		

NA: not applicable; PHWE: p value of Hardy-Weinberg equilibrium.

#### Meta-analysis of the C677T polymorphism

As shown in Table 2, the pooled data of six publications indicated no association between the *MTHFR* C677T polymorphism and thyroid diseases in any of the five comparison models. However, subgroup analyses stratified by thyroid function showed a significant association of the *MTHFR* C677T polymorphism with hypothyroidism (TT *vs.* CC+CT/recessive model: OR = 2.07, 95% CI: 1.02-4.20, P = 0.04; TT *vs.* CC/ homozygote model: OR = 2.35, 95% CI: 1.13-4.86, P = 0.02) (Figure 2B and Supplemental Figures). No relationship was found between the *MTHFR* C677T polymorphism and hyperthyroidism, even when reanalyzed by ethnicity (Table 2, Figure 2A and Supplemental Figures).

#### Meta-analysis of the A1298C polymorphism

In the analysis of the *MTHFR* A1298C polymorphism, only the recessive model comparison of a total of five

publications reached a significant difference (CC vs. AC+AA/recessive model: OR = 0.62, 95% CI: 0.38-0.99, P = 0.05). Subgroup analysis stratified by ethnicity showed a significant difference in Caucasians (CC vs. AC+AA/ recessive model: OR = 0.47, 95% CI: 0.27-0.81 P = 0.007; AC vs. AA/heterozygote model: OR = 1.92, 95% CI: 1.11-3.32, P = 0.02) (Figure 3A and Supplemental Figures). There was a significant association of MTHFR A1298C with hypothyroidism in subgroup analyses stratified by thyroid function (C vs. A/allele model: OR = 0.63, 95% CI: 0.44-0.92, P = 0.02; CC vs. AC+AA/ recessive model: OR = 0.42, 95% CI: 0.22-0.79, P = 0.007; CC vs. AA/homozygote model: OR = 0.43, 95% CI: 0.25-0.85, P = 0.02) (Figure 3B and Supplemental Figures), but no relationship was found between MTHFR A1298C and hyperthyroidism (Table 3).

#### Publication bias and sensitivity analysis

Begg's funnel plot and Egger's test were performed to evaluate the publication bias of the literature. As shown in Figure 4, the shape of the funnel plots was symmetrical in the comparison of the allele model (T *vs.* C). Then, Egger's test was adopted to provide statistical evidence of the funnel plot symmetry. The results still

showed no publication bias (P = 0.152). The results of sensitivity analysis demonstrated no significant effect of an individual study on the overall pooled OR, indicating the reliability of the results (Supplemental Figures).

Diseases	Comparison models	Studies/ publications	l <sup>2</sup>	Model	OR (95% CI)	Р
Thyroid disorders			0	verall		
	T <i>vs.</i> C	8/6	77%	Random	1.36 (0.92, 2.01)	0.12
	TT+CT vs. CC	8/6	79%	Random	1.34 (0.76, 2.39)	0.31
	TT vs. CC+CT	8/6	44%	Fixed	1.29 (0.97, 1.71)	0.08
	TT vs. CC	8/6	64%	Random	1.40 (0.72, 2.71)	0.32
	CT vs. CC	8/6	76%	Random	1.17 (0.65, 2.08)	0.60
			A	sians		
	T <i>vs.</i> C	4/3	70%	Random	1.10 (0.78, 1.57)	0.59
	TT+CT vs. CC	4/3	79%	Random	0.98 (0.51, 1.85)	0.94
	TT vs. CC+CT	4/3	65%	Random	1.29 (0.72, 2.31)	0.39
	TT vs. CC	4/3	76%	Random	1.16 (0.51, 2.65)	0.73
	CT vs. CC	4/3	79%	Random	0.89 (0.45, 1.78)	0.75
			Cau	casians		
	T <i>vs.</i> C	4/3	86%	Random	2.54 (0.67, 9.59)	0.17
	TT+CT vs. CC	4/3	85%	Random	2.54 (0.56,11.48)	0.22
	TT vs. CC+CT	4/3	36%	Fixed	1.58 (0.81, 3.10)	0.18
	TT vs. CC	4/3	60%	Random	3.09 (0.53, 18.16)	0.21
	CT vs. CC	4/3	80%	Random	2.03 (0.49, 8.41)	0.33
Hyperthyroidism			0	verall		
	T <i>vs.</i> C	3/3	85%	Random	0.86 (0.48, 1.53)	0.60
	TT+CT vs. CC	3/3	79%	Random	0.65 (0.31, 1.33)	0.24
	TT vs. CC+CT	3/3	68%	Random	1.25 (0.60, 2.59)	0.55
	TT vs. CC	3/3	80%	Random	0.93 (0.33, 2.62)	0.88
	CT vs. CC	3/3	66%	Random	0.57 (0.30, 1.06)	0.07
			A	sians		
	T <i>vs.</i> C	2/2	85%	Random	1.11 (0.61, 2.00)	0.73
	TT+CT vs. CC	2/2	84%	Random	0.83 (0.33, 2.07)	0.69
	TT vs. CC+CT	2/2	78%	Random	1.58 (0.66, 3.80)	0.31
	TT vs. CC	2/2	88%	Random	1.25 (0.30, 5.19)	0.76
	CT vs. CC	2/2	74%	Random	0.69 (0.32, 1.48)	0.34
Hypothyroidism			Overall (a	l Caucasians)		
	T <i>vs.</i> C	3/3	77%	Random	2.80 (0.99, 7.96)	0.05*
	TT+CT vs. CC	3/3	73%	Random	2.88 (0.91, 9.14)	0.07
	TT vs. CC+CT	3/3	5%	Fixed	2.07 (1.02, 4.20)	0.04*
	TT vs. CC	3/3	35%	Fixed	2.35 (1.13, 4.86)	0.02*
	CT vs. CC	3/3	65%	Random	2.30 (0.78, 6.77)	0.13

#### Table 2. Meta-analysis of the association between MTHFR C677T polymorphism and thyroid disorders

OR: odds ratio; CI: confidence interval.

\*Indicates a significant difference at P  $\leq$  0.05.

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#### **Table 3.** Meta-analysis of the association between *MTHFR* A1298C polymorphism and thyroid disorders

Diseases	Comparison models	Studies/ publications	<b> </b> <sup>2</sup>	Model	OR (95% CI)	Р
Thyroid disorders			С	verall		
	C <i>vs.</i> A	7/5	0%	Fixed	0.94 (0.76, 1.16)	0.56
	CC+AC vs. AA	7/5	0%	Fixed	0.92 (0.71, 1.18)	0.52
	CC vs. AC+AA	7/5	43%	Fixed	0.62 (0.38, 0.99)	0.05*
	CC vs. AA	7/5	22%	Fixed	0.82 (0.49, 1.39)	0.46
	AC vs. AA	7/5	33%	Fixed	1.11 (0.85, 1.45)	0.44
			A	sians		
	C <i>vs.</i> A	4/3	0%	Fixed	1.00 (0.77, 1.30)	0.99
	CC+AC vs. AA	4/3	0%	Fixed	0.96 (0.71, 1.30)	0.81
	CC vs. AC+AA	4/3	42%	Fixed	1.49 (0.52, 4.21)	0.46
	CC vs. AA	4/3	42%	Fixed	1.46 (0.51, 4.16)	0.48
	AC vs. AA	4/3	0%	Fixed	0.94 (0.69, 1.27)	0.67
			Cau	casians		
	C <i>vs.</i> A	3/2	0%	Fixed	0.85 (0.61, 1.19)	0.35
	CC+AC vs. AA	3/2	0%	Fixed	0.82 (0.51, 1.31)	0.41
	CC vs. AC+AA	3/2	0%	Fixed	0.47 (0.27, 0.81)	0.007*
	CC vs. AA	3/2	0%	Fixed	0.67 (0.36, 1.23)	0.20
	AC vs. AA	3/2	0%	Fixed	1.92 (1.11, 3.32)	0.02*
Hyperthyroidism			C	verall		
	C <i>vs.</i> A	3/3	0%	Fixed	1.17 (0.91, 1.52)	0.23
	CC+AC vs. AA	3/3	84%	Random	1.69 (0.69, 4.15)	0.25
	CC vs. AC+AA	3/3	64%	Random	0.87 (0.18, 4.09)	0.86
	CC vs. AA	3/3	41%	Fixed	2.14 (0.97, 4.71)	0.06
	AC vs. AA	3/3	89%	Random	1.98 (0.64, 6.17)	0.24
			Д	sians		
	C <i>vs.</i> A	2/2	0%	Fixed	1.06 (0.77, 1.46)	0.70
	CC+AC vs. AA	2/2	0%	Fixed	1.03 (0.72, 1.47)	0.86
	CC vs. AC+AA	2/2	70%	Random	1.03 (0.05, 23.15)	0.98
	CC vs. AA	2/2	69%	Random	1.04 (0.05, 22.59)	0.98
	AC vs. AA	2/2	0%	Fixed	1.00 (0.69, 1.44)	0.98
Hypothyroidism			Overall (a	ll Caucasians)		
	C <i>vs.</i> A	2/2	0%	Fixed	0.63 (0.44, 0.92)	0.02*
	CC+AC vs. AA	2/2	0%	Fixed	0.76 (0.45, 1.26)	0.29
	CC vs. AC+AA	2/2	0%	Fixed	0.42 (0.22, 0.79)	0.007*
	CC vs. AA	2/2	0%	Fixed	0.43 (0.22, 0.85)	0.02*
	AC vs. AA	2/2	0%	Fixed	1.09 (0.60, 1.96)	0.78

OR: odds ratio; CI: confidence interval.

\*Indicates a significant difference at P  $\leq$  0.05.

#### **Trial sequential analysis results**

For the *MTHFR* C677T polymorphism and susceptibility to thyroid disorders, the cumulative Z-curve neither crossed the trial sequential monitoring boundary nor reached the RIS (Figure 5A, 5B and Supplemental Figures); therefore, the result is

inconclusive, and large-scale studies are warranted. For the *MTHFR* A1298C polymorphism and hypothyroidism susceptibility, the final Z-value crossed the conventional threshold and the O'Brien-Fleming boundary (Figure 5C); therefore, the meta-analysis result was conclusive.

4	Case		Contr	ol		Odds Ratio		Odds Ratio				
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl		M-H, Random, 95% Cl				
1.7.1 Asians												
Arakcawa-GD+HT 2012	218	558	61	168	20.7%	1.12 [0.79, 1.61]						
Lee-GD 2016	119	244	77	200	20.3%	1.52 [1.04, 2.22]						
Mao-GD 2010	208	398	267	470	22.4%	0.83 [0.64, 1.09]		-=-{				
Subtotal (95% CI)		1200		838	63.4%	1.10 [0.78, 1.57]		<b>•</b>				
Total events	545		405									
Heterogeneity: Tau <sup>2</sup> = 0.07; Chi <sup>2</sup>	= 6.68, df	= 2 (P =	= 0.04); 12	= 70%								
Test for overall effect: Z = 0.54 (F	9 = 0.59)											
1.7.2 Caucasians												
Abu-Hassan-hyper+hypo 2019	86	328	56	198	20.0%	0.90 [0.61, 1.34]						
Kvaratskhelia-hypo 2020	21	68	4	58	8.0%	6.03 [1.93, 18.83]						
Kvaratskhelia-SCH 2017	16	38	6	38	8.6%	3.88 [1.31, 11.47]		<b>-</b>				
Subtotal (95% CI)		434		294	36.6%	2.54 [0.67, 9.59]						
Total events	123		66									
Heterogeneity: Tau <sup>2</sup> = 1.16; Chi <sup>2</sup>	= 14.28, 0	lf = 2 (F	P = 0.000	3); l² = 8	36%							
Test for overall effect: Z = 1.38 (F	9 = 0.17)											
Total (95% CI)		1634		1132	100.0%	1.36 [0.92, 2.01]		•				
Total events	668		471									
Heterogeneity: Tau <sup>2</sup> = 0.16; Chi <sup>2</sup>	= 21.74, 0	lf = 5 (F	9 = 0.0000	6); l² = 7	77%		H-01		100			
Test for overall effect: Z = 1.54 (F	9 = 0.12)						0.01	U.I I 1U decreased risk increased risk	100			
Test for subaroup differences: C	hi <sup>2</sup> = 1.42	df = 1	(P = 0.23	), l <sup>z</sup> = 2	9.8%			uecieaseu lisk incleaseu lisk				



Figure 2. Association between the *MTHFR* C677T polymorphism and hypothyroidism risk (allele model: T vs. C). A Total analysis and subgroup analyses stratified by ethnicity; B Subgroup analyses stratified by thyroid function.

### DISCUSSION

The meta-analysis of eight studies showed that there was no association between the *MTHFR* C677T polymorphism and thyroid disorders, neither in the total pooled analysis nor in subgroup analyses stratified by ethnicity. However, subgroup analyses by thyroid function indicated that the C677T variant increased the risk of hypothyroidism, although more studies

are needed to confirm this result. Previous studies observed that the plasma levels of total homocysteine (tHcy) increased in hypothyroidism (19,20); in addition, patients with thyroid diseases always had an associated increased vascular risk (38,39). According to the above results, we can easily associate the elevated tHcy concentrations with the underlying *MTHFR* C677T polymorphism. However, it is difficult to

Α		Cas	е	Contr	ol		Odds Ratio	Odds Ratio
	Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	M-H, Fixed, 95% Cl
	6.5.1 Asians							
	Arakcawa-GD+HT 2012	106	542	28	128	19.8%	0.87 [0.54, 1.39]	
	Lee-GD 2016	34	244	30	200	15.4%	0.92 [0.54, 1.56]	
	Mao-GD 2010	53	372	59	470	24.2%	1.16 [0.78, 1.72]	
	Subtotal (95% CI)		1158		798	59.4%	1.00 [0.77, 1.30]	<b>•</b>
	Total events	193		117				
	Heterogeneity: Chi2 = 0.96, df = 2	(P = 0.62	); I <sup>2</sup> = 0	%				
	Test for overall effect: Z = 0.01 (P	= 0.99)						
	6.5.2 Caucasians							
	Abu-Hassan-hyper+hypo 2019	143	328	93	196	35.6%	0.86 (0.60, 1.22)	
	Kvaratskhelia-hvpo 2020	10	68	10	58	5.0%	0.83 [0.32, 2.15]	
	Subtotal (95% CI)		396		254	40.6%	0.85 [0.61, 1.19]	
	Total events	153		103			• • • •	
	Heterogeneity: Chi <sup>2</sup> = 0.00, df = 1	(P = 0.95	): $ ^2 = 0$	%				
	Test for overall effect: Z = 0.94 (P	= 0.35)						
	Total (95% CI)		1554		1052	100.0%	0.94 [0.76, 1.16]	•
	Total events	346		220				-
	Heterogeneity $Chi^2 = 1.50$ df = 4	(P = 0.83	$1 \cdot 1^2 = 0$	%				
	Test for overall effect: $7 = 0.59$ (P	= 0.56)	/					0.2 0.5 1 2 5
	Test for subaroup differences: Cl	ni² = 0.53.	df = 1	(P = 0.47	). I² = 0	%		decreased risk increased risk
B		Case		Control			Odds Ratio	Odds Ratio
	Study or Subgroup Eve	ents Tot	al Ev	ents To	tal W	eight M	H. Fixed, 95% CI	M.H. Fixed, 95% Cl



#### Test for subaroup differences: Chi<sup>2</sup> = 7.13. df = 1 (P = 0.008). l<sup>2</sup> = 86.0%

Figure 3. Association between the *MTHFR*A1298C polymorphism and hypothyroidism risk (allele model: C vs. A). A Total analysis and subgroup analyses stratified by ethnicity; **B** Subgroup analyses stratified by thyroid function.



Figure 4. Begg's funnel plot for the odds ratio of the MTHFR C677T allele frequency comparison (T vs. C) in thyroid dysfunction.

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Figure 5. Trial sequential analysis of the total analysis and hypothyroidism subgroup analysis in the allele model. A Total analysis of the *MTHFR* C677T polymorphism; B Hypothyroidism subgroup analysis of the *MTHFR* C677T polymorphism; C Hypothyroidism subgroup analysis of the *MTHFR* A1298C polymorphism.

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explain why the plasma levels of tHcy were normalized after thyroid hormone replacement therapy (19,20). In the past few decades, a large body of studies have been conducted worldwide to elucidate the molecular mechanism of the association between MTHFR gene polymorphisms and other diseases. Ueland and cols. summarized that the relationship between the MTHFR C677T polymorphism and disease involves two aspects. First, the disease might influence tHcy concentrations, and effect modification might occur from the MTHFR polymorphism. Second, the genotype might be associated with disease risk due to the altered metabolism of folates (40). Moreover, Hustad and cols. supported that thyroid status affects the phenotypic expression of the MTHFR C677T polymorphism, possibly by modifying the availability of flavin cofactors (27). We know that the C to T mutation results in thermolability of MTHFR, which leads to a higher dissociation rate of flavin adenine dinucleotide (FAD), the cofactor of MTHFR. Thyroid hormones (free thyroxine and free triiodothyronine) increase the activity of enzymes involved in riboflavin metabolism, particularly riboflavin kinase (RK), and thereby augment the synthesis of FAD (27). Therefore, thyroid hormones, riboflavin, folate and MTHFR gene polymorphisms all play a role in homocysteine metabolism, but each of them provides a small contribution. This can also explain why not all MTHFR SNP C677T carriers demonstrate hyperhomocysteinemia, unless in conditions with low concentrations of thyroid hormones, riboflavin or folate (21,41-43).

With regard to the MTHFR A1298C polymorphism, subgroup analyses by thyroid function indicated that the MTHFR A1298C polymorphism decreased the risk of hypothyroidism. The results were in accordance with the studies of Abu-Hassan and cols. and Kvaratskhelia and cols. Some studies (5,6,44) also found that the MTHFR A1298C polymorphism is in linkage disequilibrium (LD) with the C677T polymorphism, and Abu-Hassan and cols. suggested that the interaction of the SNPs within haplotypes might act as a major determinant of disease susceptibility in comparison with the single polymorphisms in the MTHFR gene among hypothyroidism cases. They believed that carriers of the CC (677C-1298C) and TA (677T-1298A) haplotypes had significantly lower risks of hypothyroidism, whereas those with TC (677T-1298C) haplotypes had a higher likelihood of having hypothyroidism (6). Lee and cols. reported that the MTHFR 677CT/1298AA genotype decreased the risk of ophthalmopathy in patients with GD, but the *MTHFR* 677T/1298A haplotype increased the risk of GD without ophthalmopathy. Therefore, more studies examining the relationship of the C677T and A1298C haplotypes with thyroid dysfunction are required. Besides, previous studies reported that the *MTHFR* C677T and A1298C polymorphisms had different and even opposite effect on cell metabolism and DNA methylation (45,46). These results suggested that different polymorphisms might have different influences on thyroid function because of diverse pathogenesis, except for reduced enzyme activity. However, more basic researches are needed to explore the underlying molecular mechanism.

Other than the two most common *MTHFR* gene polymorphisms, Mao and cols. also investigated the relationship between GD and another *MTHFR* SNP-G1793A (rs2274976) — another mutation occurs at position 1,793 and results in alteration of the translation of an arginine to a glutamine. They observed that individuals with the variant genotypes (GA+AA) appeared to have a slightly higher risk of GD, but it was not statistically significant (30). Compared with the two common SNPs, the frequencies of the G1793A variant genotypes were very low in both the cases and the controls. In this context, many more participants are needed to reveal the potential relationship.

There are a few limitations in our meta-analysis. First, the numbers of included studies for our metaanalysis were relatively small, especially in the subgroup analyses. Second, our results were based on unadjusted estimates, and some other covariants, including age, sex, environmental factors, and other lifestyle factors, were not controlled in our analysis. Moreover, several included studies were inconsistent with HWE in the controls. The studies of Abu-Hassan and cols. in 2019 and Kvaratskhelia and cols. in 2020 included females only, which would result in nonconformity with HWE. Mao and cols. stated that conformity to HWE among each population was performed in their article, but the present data suggested a departure from HWE of the MTHFR C677T SNP. Hence, more high-quality studies on the association between MTHFR gene polymorphisms and thyroid disease are needed, and meta-analysis for each individual thyroid disease (HT, GD, hyperthyroidism, and hypothyroidism) is necessary to elucidate the true relationship with MTHFR gene polymorphisms.

In conclusion, the present meta-analysis suggests that the C677T variant of the *MTHFR* gene increases the risk of hypothyroidism, while the *MTHFR* A1298C variation may protect patients against hypothyroidism. However, further well-designed, large-sample-size studies are warranted to confirm the association between the *MTHFR* C677T polymorphism and hypothyroidism.

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### SUPPLEMENTAL INFORMATION

Association of methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphisms (C677T and A1298C) with thyroid dysfunction: A meta-analysis and trial sequential analysis

Rui Yang\*, Danhua Pu\*, Rongrong Tan, Jie Wu

### **Supplemental tables**

#### Table S1. Newcastle-Ottawa Scale for assessing the quality of studies

		Selec	ction				Exposure			
Study	1) Adequate definition of the cases	2) Representativeness of the cases	3) Selection of the controls	4) Definition of the controls	Comparability of the cases and controls on the basis of the design or analysis	1) Ascertainment of exposure	<ol> <li>Same method of ascertainment for the cases and controls</li> </ol>	3) Non-response rate	Scores	
Pan 2004	*	#	#	#	#	*	*	*	4	
Mao 2010	*	*	*	*	**	*	*	*	9	
Arakawa 2012	*	*	#	*	#	*	*	*	6	
Lee 2016	*	*	#	*	**	*	*	*	8	
Kvaratskhelia 2017	*	#	#	*	*	*	*	*	6	
Abu-Hassan 2019	#	*	*	#	*	*	*	*	6	
Kvaratskhelia 2020	*	*	#	*	*	*	*	*	7	

**Supplemental figures** 

The association between the *MTHFR* C677T polymorphism and the risk of thyroid diseases **1. Allele model: T vs. C** 

- 1.1 Forest plots
- (1) Total analysis and subgroup analyses stratified by ethnicity see Figure 2A
- (2) Subgroup analyses stratified by thyroid function see Figure 2B
- 1.2 Figure of sensitivity analysis



### 1.3 Figure of trial sequential analysis

(1) Total analysis (A) and subgroup analyses stratified by ethnicity (B Assians and C Caucassians)

A Total analysis see Figure 5A

**B** Assians



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### C Caucassians



(2) Subgroup analyses stratified by thyroid function (A hyperthyroidism and B hypothyroidism) A Hyperthyroidism



**B** Hypothyroidism see Figure 5B

### 2. Dominant model: TT+TC vs. CC

### 2.1 Forest plots

(1) Total analysis and subgroup analyses stratified by ethnicity

	Cas	е	Contr	ol		Odds Ratio	Odds Ratio						
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl						
2.4.1 Asians													
Arakcawa-GD+HT 2012	180	279	48	84	19.9%	1.36 [0.83, 2.24]	+						
Lee-GD 2016	87	122	65	100	19.0%	1.34 [0.76, 2.36]							
Mao-GD 2010	148	199	199	235	20.1%	0.52 [0.33, 0.85]							
Subtotal (95% CI)		600		419	59.1%	0.98 [0.51, 1.85]	<b>•</b>						
Total events	415		312										
Heterogeneity: Tau <sup>2</sup> = 0.25; Chi <sup>2</sup>	= 9.38, df	= 2 (P :	= 0.009);	<sup>2</sup> = 799	%								
Test for overall effect: Z = 0.07 (P	= 0.94)												
2.4.2 Caucasians													
Abu-Hassan-hyper+hypo 2019	64	164	44	99	19.8%	0.80 (0.48, 1.33)							
Kvaratskhelia-hvpo 2020	18	34	4	29	11.1%	7.03 [2.01, 24.59]							
Kvaratskhelia-SCH 2017	11	19	5	19	10.1%	3.85 [0.98, 15.12]							
Subtotal (95% CI)		217		147	40.9%	2.54 [0.56, 11.48]							
Total events	93		53			•							
Heterogeneity: Tau <sup>2</sup> = 1.47; Chi <sup>2</sup>	= 12.96. d	f = 2 (F)	P = 0.002	: I <sup>2</sup> = 85	5%								
Test for overall effect: Z = 1.21 (P	= 0.22)		,										
	/												
Total (95% CI)		817		566	100.0%	1.34 [0.76, 2.39]							
Total events	508		365										
Heterogeneity: Tau <sup>2</sup> = 0.37; Chi <sup>2</sup>	= 23.31, d	f = 5 (F	= 0.0003	3); l² = 7	79%								
Test for overall effect: Z = 1.01 (P	= 0.31)				N. N. 12230		U.U5 U.2 1 5 20						
Toot for outparoun differences: C	hiz - 1 21	df - 1	/D - 0.25	12-2	2.00%		decreased risk increased risk						

Test for subaroup differences:  $Chi^2 = 1.31$ . df = 1 (P = 0.25).  $I^2 = 23.9\%$ 

(2) Subgroup analyses stratified by thyroid function

	Cas	е	Control			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl
2.3.1 hyperthyroidism							
Abu-Hassan-hyper 2019	15	66	44	99	30.4%	0.37 [0.18, 0.74]	
Lee-GD 2016	87	122	65	100	33.7%	1.34 [0.76, 2.36]	-+
Mao-GD 2010	148	199	199	235	35.9%	0.52 [0.33, 0.85]	
Subtotal (95% CI)		387		434	100.0%	0.65 [0.31, 1.33]	
Total events	250		308				
Heterogeneity: Tau <sup>2</sup> = 0.32;	Chi <sup>2</sup> = 9.	53, df =	2 (P = 0.	.009); lª	²= 79%		
Test for overall effect: Z = 1.	.18 (P = 0	.24)					
2.3.2 hypothyroidism							
Abu-Hassan-hypo 2019	49	98	44	99	41.9%	1.25 [0.71, 2.19]	
Kvaratskhelia-hypo 2020	18	34	4	29	30.0%	7.03 [2.01, 24.59]	
Kvaratskhelia-SCH 2017	11	19	5	19	28.1%	3.85 [0.98, 15.12]	
Subtotal (95% CI)		151		147	100.0%	2.88 [0.91, 9.14]	
Total events	78		53				
Heterogeneity: Tau <sup>2</sup> = 0.75;	Chi <sup>2</sup> = 7.	37, df=	2 (P = 0.	.03); l² =	= 73%		
Test for overall effect: Z = 1.	.80 (P = 0	.07)					
							U.UD U.Z 1 5 ZU
To all fam and success differences	OI-17	1 00	16 4 10	0.000	17 70 44	N .	decreased lisk increased lisk

Test for subaroup differences:  $Chi^2 = 4.62$ . df = 1 (P = 0.03).  $I^2 = 78.4\%$ 

### 2.2 Figure of sensitivity analysis



### 3. Recessive model: TT vs. TC+CC

### 3.1 Forest plots

(1) Total analysis (A) and subgroup analyses stratified by ethnicity (B Asians and C Caucasians)

A Total analysis

-

	Case Control			Odds Ratio	Odds Ratio				
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	M-H, Fixed, 95% Cl		
Abu-Hassan-hyper+hypo 2019	22	164	12	99	15.3%	1.12 [0.53, 2.38]			
Arakcawa-GD+HT 2012	38	279	13	84	20.4%	0.86 [0.43, 1.71]			
Kvaratskhelia-hypo 2020	3	34	0	29	0.6%	6.56 [0.32, 132.39]			
Kvaratskhelia-SCH 2017	5	19	1	19	0.9%	6.43 [0.67, 61.47]			
Lee-GD 2016	32	122	12	100	11.5%	2.61 [1.26, 5.39]			
Mao-GD 2010	60	199	68	235	51.4%	1.06 [0.70, 1.60]	+		
Total (95% CI)		817		566	100.0%	1.29 [0.97, 1.71]	<b>◆</b>		
Total events	160		106						
Heterogeneity: Chi <sup>2</sup> = 9.01, df = 5	(P = 0.11	); I <sup>2</sup> = 4	4%						
Test for overall effect: Z = 1.73 (P	= 0.08)						decreased risk increased risk		

#### **B** Asians

	Cas	е	Contr	ol		Odds Ratio		Odds Ratio			
Study or Subgroup	<b>Events</b>	Total	<b>Events</b>	Total	Weight	M-H, Random, 95% Cl		M-H, Random,	95% CI		
Arakcawa-GD+HT 2012	38	279	13	84	30.2%	0.86 [0.43, 1.71]					
Lee-GD 2016	32	122	12	100	28.7%	2.61 [1.26, 5.39]		-	-		
Mao-GD 2010	60	199	68	235	41.1%	1.06 [0.70, 1.60]					
Total (95% CI)		600		419	100.0%	1.29 [0.72, 2.31]		-	•		
Total events	130		93								
Heterogeneity: Tau <sup>2</sup> = 0.17	$r; Chi^2 = 6$	5.66, df	= 2 (P = 0	0.06); l²	= 65%		0.01	0.1 1	10	100	
Test for overall effect. $Z = 0$	J.80 (P =	0.39)						decreased risk ind	creased risk		

### **C** Caucasians

	Cas	е	Contr	ol		Odds Ratio		Odds	Ratio		
Study or Subgroup	Events	Total	<b>Events</b>	Total	Weight	M-H, Fixed, 95% Cl		M-H, Fixe	d, 95% CI		
Abu-Hassan-hyper+hypo 2019	22	164	12	99	91.4%	1.12 [0.53, 2.38]		-	-		
Kvaratskhelia-hypo 2020	3	34	0	29	3.4%	6.56 [0.32, 132.39]					
Kvaratskhelia-SCH 2017	5	19	1	19	5.2%	6.43 [0.67, 61.47]		-			
Total (95% CI) Total events Heterogeneity: Chi <sup>2</sup> = 3.14, df = 2	30 (P = 0.21	217 ); I² = 3	13 :6%	147	100.0%	1.58 [0.81, 3.10]	<del>_ </del>	-+ 0.1 1	•	10	
Test for overall effect: Z = 1.34 (P	= 0.18)							decreased risk	increased	risk	

(2) Subgroup analyses stratified by thyroid function (A Hyperthyroidism and B Hypothyroidism)

A Hyperthyroidism

-

	Cas	е	Contr	ol		Odds Ratio		Odd	s Ratio		
Study or Subgroup	Events	Total	<b>Events</b>	Total	Weight	M-H, Random, 95% Cl		M-H, Ran	dom, 95%	6 CI	
Abu-Hassan-hyper 2019	5	66	12	99	23.5%	0.59 [0.20, 1.77]			-		
Lee-GD 2016	32	122	12	100	33.4%	2.61 [1.26, 5.39]				_	
Mao-GD 2010	60	199	68	235	43.1%	1.06 [0.70, 1.60]		-	+		
Total (95% CI)		387		434	100.0%	1.25 [0.60, 2.59]		-			
Total events	97		92								
Heterogeneity: Tau <sup>2</sup> = 0.27;	Chi <sup>2</sup> = 6.		L 01		+	10	100				
Test for overall effect: $Z = 0$	0.01	decreased risi	increa	sed risk	100						

### B Hypothyroidism

	Cas	е	Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	M-H, Fixed, 95% Cl
Abu-Hassan-hypo 2019	17	98	12	99	89.0%	1.52 [0.68, 3.38]	
Kvaratskhelia-hypo 2020	3	34	0	29	4.4%	6.56 [0.32, 132.39]	
Kvaratskhelia-SCH 2017	5	19	1	19	6.6%	6.43 [0.67, 61.47]	
Total (95% CI)		151		147	100.0%	2.07 [1.02, 4.20]	•
Total events	25		13				
Heterogeneity: Chi <sup>2</sup> = 2.10,	df = 2 (P :	= 0.35)					
Test for overall effect: Z = 2.	01 (P = 0	.04)	decreased risk increased risk				

### 3.2 Figure of sensitivity analysis



3.3 Figure of trial sequential analysis (only hypothyroidism-subgroup analysis)



### 4. Homozygote model: TT vs. CC

### 4.1 Forest plots

(1) Total analysis and subgroup analyses stratified by ethnicity

	Cas	е	Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl
4.4.1 Asians							
Arakcawa-GD+HT 2012	38	137	13	49	22.1%	1.06 [0.51, 2.22]	
Lee-GD 2016	32	67	12	47	20.8%	2.67 [1.18, 6.01]	
Mao-GD 2010	60	111	68	104	25.2%	0.62 [0.36, 1.08]	
Subtotal (95% CI)		315		200	68.1%	1.16 [0.51, 2.65]	-
Total events	130		93				
Heterogeneity: Tau <sup>2</sup> = 0.40; Chi <sup>2</sup> :	= 8.48, df	= 2 (P =	= 0.01); l <sup>2</sup>	= 76%			
Test for overall effect: Z = 0.35 (P	= 0.73)						
4.4.2 Caucasians							
Abu-Hassan-hyper+hypo 2019	22	122	12	67	21.4%	1.01 [0.46, 2.19]	
Kvaratskhelia-hypo 2020	3	19	0	25	4.1%	10.82 [0.52, 223.28]	
Kvaratskhelia-SCH 2017	5	13	1	15	6.4%	8.75 [0.86, 88.69]	
Subtotal (95% CI)		154		107	31.9%	3.09 [0.53, 18.16]	
Total events	30		13				
Heterogeneity: Tau <sup>2</sup> = 1.47; Chi <sup>2</sup> :	= 4.95, df	= 2 (P =	= 0.08); l <sup>2</sup>	= 60%			
Test for overall effect: Z = 1.25 (P	= 0.21)						
Total (95% CI)		469		307	100.0%	1.40 [0.72, 2.71]	-
Total events	160		106				
Heterogeneity: Tau <sup>2</sup> = 0.37; Chi <sup>2</sup> :	= 14.06, d						
Test for overall effect: Z = 1.00 (P	= 0.32)		decreased risk increased risk				
Test for subaroup differences: Cl	hi² = 0.97.	df = 1	(P = 0.33)	), $ ^2 = 0$	%		uetreased lisk increased lisk

(2) Subgroup analyses stratified by thyroid function (A and B)

### A Hyperthyroidism

	Case		Case		Control			Odds Ratio		Odds Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl		M-H, Random, 95% Cl			
Abu-Hassan-hyper 2019	5	56	12	67	28.5%	0.45 [0.15, 1.36]					
Lee-GD 2016	32	67	12	47	33.6%	2.67 [1.18, 6.01]					
Mao-GD 2010	60	111	68	104	37.8%	0.62 [0.36, 1.08]					
Total (95% CI)		234		218	100.0%	0.93 [0.33, 2.62]					
Total events	97		92								
Heterogeneity: Tau <sup>2</sup> = 0.66;	Chi <sup>2</sup> = 1		1 0.05		ł						
Test for overall effect: $Z = 0$ .	.15 (P = 0	0.00	decreased risk increased risk	,							

### **B** Hypothyroidism

	Cas	е	Contr	ol		Odds Ratio		Odds Ratio		
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl		M-H, Fixed, 95% Cl		
Abu-Hassan-hypo 2019	17	66	12	67	90.5%	1.59 [0.69, 3.66]				
Kvaratskhelia-hypo 2020	3	19	0	25	3.7%	10.82 [0.52, 223.28]				$\rightarrow$
Kvaratskhelia-SCH 2017	5	13	1	15	5.8%	8.75 [0.86, 88.69]		1	-	
Total (95% CI)		98		107	100.0%	2.35 [1.13, 4.86]		-		
Total events	25		13							
Heterogeneity: Chi <sup>2</sup> = 3.06,	df = 2 (P	= 0.22)	; I² = 35%				0.02 0.1	1 1	10	50
Test for overall effect: $Z = 2$ .	.02)					de	creased risk increase	d risk		

4.2 Figure of sensitivity analysis



4.3 Figure of trial sequence analysis (only hypothyroidism-subgroup analysis)



### 5. Heterozygote model: TC vs. CC

### 5.1 Forest plots

(1) Total analysis and subgroup analyses stratified by ethnicity

	Case	e	Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl
5.4.1 Asians							
Arakcawa-GD+HT 2012	142	241	35	71	20.2%	1.48 [0.87, 2.51]	1 +
Lee-GD 2016	55	90	53	88	19.3%	1.04 [0.57, 1.89]	ı — <del>• •</del>
Mao-GD 2010	88	139	131	167	20.5%	0.47 [0.29, 0.79]	
Subtotal (95% CI)		470		326	60.0%	0.89 [0.45, 1.78]	
Total events	285		219				
Heterogeneity: Tau <sup>2</sup> = 0.30; Chi <sup>2</sup> =	9.67, df	= 2 (P =	= 0.008);	<sup>2</sup> = 799	Хо		
Test for overall effect: Z = 0.32 (P	= 0.75)						
5.4.2 Caucasians							
Abu-Hassan-hyper+hypo 2019	42	142	32	87	19.8%	0.72 [0.41, 1.27]	ı <del>−•+</del>
Kvaratskhelia-hypo 2020	15	31	4	29	11.2%	5.86 [1.65, 20.84]	
Kvaratskhelia-SCH 2017	6	14	4	18	9.0%	2.63 [0.57, 12.18]	
Subtotal (95% CI)		187		134	40.0%	2.03 [0.49, 8.41]	
Total events	63		40				
Heterogeneity: Tau <sup>2</sup> = 1.24; Chi <sup>2</sup> =	= 10.07, d	f= 2 (P	= 0.006)	; I <sup>2</sup> = 80	)%		
Test for overall effect: Z = 0.97 (P	= 0.33)						
Total (95% CI)		657		460	100.0%	1.17 [0.65, 2.08]	▲
Total events	348		259				
Heterogeneity: Tau <sup>2</sup> = 0.36; Chi <sup>2</sup> =	= 20.48, d						
Test for overall effect: Z = 0.52 (P	= 0.60)						decreased risk increased risk
Test for subaroup differences: Cl	ni² = 1.03.	df = 1	(P = 0.31	),  ² = 3	.0%		Gettedseu lisk intredseu lisk

### (2) Subgroup analyses stratified by thyroid function

	Case	e	Control		Odds Ratio		Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl
5.3.1 hyperthyroidism							
Abu-Hassan-hyper 2019	10	61	32	87	17.4%	0.34 [0.15, 0.75]	
Lee-GD 2016	55	90	53	88	19.6%	1.04 [0.57, 1.89]	<b>+</b>
Mao-GD 2010	88	139	131	167	20.6%	0.47 [0.29, 0.79]	
Subtotal (95% CI)		290		342	57.6%	0.57 [0.30, 1.06]	
Total events	153		216				
Heterogeneity: Tau <sup>2</sup> = 0.20;	Chi <sup>2</sup> = 5.9	95, df=	2 (P = 0.	05); l² =	= 66%		
Test for overall effect: Z = 1.	79 (P = 0	.07)					
5.3.2 hypothyroidism							
Abu-Hassan-hypo 2019	32	81	32	87	19.4%	1.12 [0.60, 2.09]	
Kvaratskhelia-hypo 2020	15	31	4	29	12.6%	5.86 [1.65, 20.84]	
Kvaratskhelia-SCH 2017	6	14	4	18	10.4%	2.63 [0.57, 12.18]	
Subtotal (95% CI)		126		134	42.4%	2.30 [0.78, 6.77]	
Total events	53		40				
Heterogeneity: Tau <sup>2</sup> = 0.58;	Chi <sup>2</sup> = 5.0	66, df=	2 (P = 0.	06); l² =	= 65%		
Test for overall effect: Z = 1.	51 (P = 0	.13)					
Total (95% CI)		416		476	100.0%	1.01 [0.52, 1.96]	-
Total events	206		256				
Heterogeneity: Tau <sup>2</sup> = 0.49;	Chi <sup>2</sup> = 22	2.17, df	= 5 (P = 1	0.0005)	)		
Test for overall effect: Z = 0.	03 (P = 0	.98)					decreased risk increased risk
To al fam and sugar differences		100	10 4 10	0.00	12 20 44	v.	uecieaseu lisk illiteaseu lisk

Test for subaroup differences:  $Chi^2 = 4.85$ . df = 1 (P = 0.03).  $l^2 = 79.4\%$ 

5.2 Figure of sensitivity analysis



## The association between the MTHFR A1298C polymorphism and the risk of thyroid diseases

### 1. Allele model: C vs. A

- 1.1 Forest plots
- (1) Total analysis and subgroup analyses stratified by ethnicity see Figure 3A
- (2) Subgroup analyses stratified by thyroid function see Figure 3B
- 1.2 Figure of sensitivity analysis



### 1.3 Figure of trial sequential analysis

(1) Total analysis (A) and subgroup analyses stratified by ethnicity (B Assians and C Caucassians) A Total analysis







### C Caucassians



(2) Subgroup analyses stratified by thyroid function (A hyperthyroidism and B hypothyroidism) A Hyperthyroidism



### **B** Hypothyroidism see Figure 5C

### 2. Dominant model: CC+CA vs. AA

2.1 Forest plots

(1) Total analysis and subgroup analyses stratified by ethnicity

	Cas	e	Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	M-H, Fixed, 95% CI
7.3.1 Asians							
Arakcawa-GD+HT 2012	101	271	27	64	21.7%	0.81 [0.47, 1.42]	
Lee-GD 2016	34	122	28	100	17.6%	0.99 [0.55, 1.79]	
Mao-GD 2010	47	186	57	235	29.9%	1.06 [0.68, 1.65]	
Subtotal (95% CI)		579		399	69.2%	0.96 [0.71, 1.30]	-
Total events	182		112				
Heterogeneity: Chi <sup>2</sup> = 0.53, df = 2	(P = 0.77	); l² = 0	%				
Test for overall effect: Z = 0.24 (P	= 0.81)						
7.3.2 Caucasians							
Abu-Hassan-hyper+hypo 2019	93	164	61	98	26.2%	0.79 [0.48, 1.33]	
Kvaratskhelia-hypo 2020	8	34	7	29	4.6%	0.97 [0.30, 3.09]	
Subtotal (95% CI)		198		127	30.8%	0.82 [0.51, 1.31]	
Total events	101		68				
Heterogeneity: Chi <sup>2</sup> = 0.09, df = 1	(P = 0.76	); l² = 0	%				
Test for overall effect: Z = 0.83 (P	= 0.41)						
Total (95% CI)		777		526	100.0%	0.92 [0.71, 1.18]	-
Total events	283		180				
Heterogeneity: Chi <sup>2</sup> = 0.94, df = 4	(P = 0.92	); l² = 0	%				
Test for overall effect: Z = 0.65 (P	= 0.52)						Favours (experimental) Favours (control)
Test for subaroup differences: Cl	ni² = 0.33.	df = 1 (	(P = 0.57	), I <sup>2</sup> = 0	%		r avours (experimental) i avours (control)

(2) Subgroup analyses stratified by thyroid function (A hyperthyroidism and B hypothyroidism)

### A Hyperthyroidism

	Cas	е	Control		Odds Ratio			Odds Ratio	
Study or Subgroup	<b>Events</b>	Total	<b>Events</b>	Total	Weight	M-H, Random, 95% Cl		M-H, Random, 95% Cl	
Abu-Hassan-hyper 2019	60	66	61	98	28.3%	6.07 [2.39, 15.42]			
Lee-GD 2016	34	122	28	100	34.7%	0.99 [0.55, 1.79]			
Mao-GD 2010	47	186	57	235	37.1%	1.06 [0.68, 1.65]			
Total (95% CI)		374		433	100.0%	1.69 [0.69, 4.15]			
Total events	141		146						
Heterogeneity: Tau <sup>2</sup> = 0.51;	Chi <sup>2</sup> = 12	1 05							
Test for overall effect: $Z = 1$ .	15 (P = 0	.25)					0.00	decreased risk increased risk	

### **B** Hypothyroidism

	Case		Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% Cl
Abu-Hassan-hypo 2019	53	98	61	98	82.9%	0.71 [0.40, 1.26]	
Kvaratskhelia-hypo 2020	8	34	7	29	17.1%	0.97 [0.30, 3.09]	
Total (95% CI)		132		127	100.0%	0.76 [0.45, 1.26]	
Total events	61		68				
Heterogeneity: Chi² = 0.21, Test for overall effect: Z = 1	df = 1 (P .06 (P = 0	= 0.65) .29)	; l² = 0%				0.2 0.5 1 2 5 decreased risk increased risk

### 2.2 Figure of sensitivity analysis



### 3. Recessive model: CC vs. CA+AA

### 3.1 Forest plots

(1) Total analysis and subgroup analyses stratified by ethnicity

	Case		Contr	ol		Odds Ratio	Odds Ratio			
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	M-H, Fixed, 95% Cl			
8.3.1 Asians										
Arakcawa-GD+HT 2012	5	271	1	64	3.8%	1.18 [0.14, 10.32]				
Lee-GD 2016	0	122	2	100	6.5%	0.16 [0.01, 3.39]	← • • • • • • • • • • • • • • • • • • •			
Mao-GD 2010	6	186	2	235	4.1%	3.88 [0.77, 19.47]				
Subtotal (95% CI)		579		399	14.4%	1.49 [0.52, 4.21]				
Total events	11		5							
Heterogeneity: Chi <sup>2</sup> = 3.45, df = 2	(P = 0.18)	;   <sup>2</sup> = 4	2%							
Test for overall effect: Z = 0.74 (P	= 0.46)									
8.3.2 Caucasians							_			
Abu-Hassan-hyper+hypo 2019	30	164	32	98	78.3%	0.46 [0.26, 0.82]				
Kvaratskhelia-hypo 2020	2	34	3	29	7.3%	0.54 [0.08, 3.49]				
Subtotal (95% CI)		198		127	85.6%	0.47 [0.27, 0.81]	<b>•</b>			
Total events	32		35							
Heterogeneity: Chi <sup>2</sup> = 0.03, df = 1	(P = 0.87)	; l² = 0	%							
Test for overall effect: Z = 2.69 (P	= 0.007)									
Total (95% CI)		777		526	100.0%	0.62 [0.38, 0.99]	$\bullet$			
Total events	43		40							
Heterogeneity: Chi <sup>2</sup> = 7.08, df = 4	(P = 0.13)	;   <sup>2</sup> = 4	3%							
Test for overall effect: Z = 1.99 (P	= 0.05)						Eavours [evnerimental] Eavours [control]			
Test for subaroup differences: C	hi² = 3.67.	df = 1 (	(P = 0.06	),   <sup>2</sup> = 7	2.8%		ravous (experimental) Favous (control)			

(2) Subgroup analyses stratified by thyroid function (A hyperthyroidism and B hypothyroidism) A Hyperthyroidism

	Cas	е	Contr	ol		Odds Ratio		Odds Ratio
Study or Subgroup	Events	Total	<b>Events</b>	Total	Weight	M-H, Random, 95% Cl		M-H, Random, 95% Cl
Abu-Hassan-hyper 2019	14	66	32	98	48.4%	0.56 [0.27, 1.15]		
Lee-GD 2016	0	122	2	100	17.5%	0.16 [0.01, 3.39]	←	• •
Mao-GD 2010	6	186	2	235	34.1%	3.88 [0.77, 19.47]		
Total (95% CI) Total events	20	374	36	433	100.0%	0.87 [0.18, 4.09]		
Heterogeneity: Tau <sup>2</sup> = 1.16 Test for overall effect: Z = 0	; Chi <sup>2</sup> = 5. .18 (P = 0	l 0.02	0.1 1 10 50 decreased risk increased risk					

### **B** Hypothyroidism

	Case		Control		Odds Ratio		Odds Ratio			
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl		M-H, Fixe	d, 95% Cl	
Abu-Hassan-hypo 2019	16	98	32	98	89.8%	0.40 [0.20, 0.80]				
Kvaratskhelia-hypo 2020	2	34	3	29	10.2%	0.54 [0.08, 3.49]	20	•		
Total (95% CI)		132		127	100.0%	0.42 [0.22, 0.79]		•		
Total events	18		35							
Heterogeneity: Chi <sup>2</sup> = 0.09,	df = 1 (P :	= 0.77)	² = 0%			1 0.05	0.2			
Test for overall effect: $Z = 2$ .					0.00	decreased risk	increased risk	20		

3.2 Figure of sensitivity analysis



3.3 Figure of trial sequence analysis (A Caucasians and B hypothyroidism-subgroup analysis) A Caucasians



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### **B** Hypothyroidism



### 4. Homozygote model: CC vs. AA

### 4.1 Forest plots

-

(1) Total analysis and subgroup analyses stratified by ethnicity

	Case		Control		Odds Ratio		Odds Ratio			
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl		M-H, Fixe	d, 95% Cl	
9.3.1 Asians										
Arakcawa-GD+HT 2012	5	175	1	38	5.2%	1.09 [0.12, 9.59]				
Lee-GD 2016	0	88	2	74	8.8%	0.16 [0.01, 3.47]	←	-		
Mao-GD 2010	6	145	2	180	5.6%	3.84 [0.76, 19.33]		-	•	-
Subtotal (95% CI)		408		292	19.6%	1.46 [0.51, 4.16]				
Total events	11		5							
Heterogeneity: Chi2 = 3.42, df = 2	(P = 0.18	); $ ^2 = 4$	2%							
Test for overall effect: Z = 0.70 (P	= 0.48)									
9.3.2 Caucasians								_		
Abu-Hassan-hyper+hypo 2019	30	81	32	69	70.9%	0.68 [0.35, 1.31]				
Kvaratskhelia-hypo 2020	2	28	3	25	9.6%	0.56 [0.09, 3.69]	-			
Subtotal (95% CI)		109		94	80.4%	0.67 [0.36, 1.23]		-	•	
Total events	32		35							
Heterogeneity: Chi2 = 0.03, df = 1	(P = 0.85	); I <sup>2</sup> = 0	%							
Test for overall effect: Z = 1.29 (P	= 0.20)									
Total (95% CI)		517		386	100.0%	0.82 [0.49, 1.39]		-		
Total events	43		40							
Heterogeneity: Chi2 = 5.11, df = 4	Heterogeneity: Chi <sup>2</sup> = 5.11, df = 4 (P = 0.28); l <sup>2</sup> = 22%									
Test for overall effect: Z = 0.74 (P	= 0.46)						0.02 U	lacrossed risk	increased risk	50
Test for subgroup differences: Chi <sup>2</sup> = 1.59. df = 1 (P = 0.21). l <sup>2</sup> = 37.1%										

(2) Subgroup analyses stratified by thyroid function

	Case	e	Control		Odds Ratio			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl		M-H, Fixed, 95% Cl
9.2.1 hyperthyroidism								
Abu-Hassan-hyper 2019	14	20	32	69	49.5%	2.70 [0.93, 7.84]		+-■
Lee-GD 2016	0	88	2	74	30.9%	0.16 [0.01, 3.47]	•	
Mao-GD 2010	6	145	2	180	19.6%	3.84 [0.76, 19.33]		
Subtotal (95% CI)		253		323	100.0%	2.14 [0.97, 4.71]		
Total events	20		36					
Heterogeneity: Chi <sup>2</sup> = 3.41,	df = 2 (P =	= 0.18)	<sup>2</sup> = 41%					
Test for overall effect: Z = 1.	89 (P = 0.	.06)						
9.2.2 hypothyroidism								_
Abu-Hassan-hypo 2019	16	61	32	69	88.3%	0.41 [0.20, 0.86]		
Kvaratskhelia-hypo 2020	2	28	3	25	11.7%	0.56 [0.09, 3.69]		
Subtotal (95% CI)		89		94	100.0%	0.43 [0.22, 0.85]		-
Total events	18		35					
Heterogeneity: Chi <sup>2</sup> = 0.09,	df = 1 (P =	= 0.76)	; l² = 0%					
Test for overall effect: Z = 2.	41 (P = 0.	.02)						
							1 02	
							0.02	decreased risk increased risk

Test for subaroup differences: Chi<sup>2</sup> = 9.03. df = 1 (P = 0.003). l<sup>2</sup> = 88.9%

4.2 Figure of sensitivity analysis



### 4.3 Figure of trial sequence analysis (only hypothyroidism-subgroup analysis)



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### 5. Heterozygote model: CA vs. AA

### 5.1 Forest plots

(1) Total analysis and subgroup analyses stratified by ethnicity



(2) Subgroup analyses stratified by thyroid function (A hyperthyroidism and B hypothyroidism)

#### A Hyperthyroidism

	Cas	е	Control			Odds Ratio	Odds Ratio			
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl			
Abu-Hassan-hyper 2019	46	52	29	66	29.7%	9.78 [3.67, 26.06]				
Lee-GD 2016	34	122	26	98	34.5%	1.07 [0.59, 1.95]				
Mao-GD 2010	41	180	55	233	35.9%	0.95 [0.60, 1.51]				
Total (95% CI)		354		397	100.0%	1.98 [0.64, 6.17]				
Total events	121		110							
Heterogeneity: Tau <sup>2</sup> = 0.88;	Chi <sup>2</sup> = 18	8.62, df	= 2 (P <	0.0001	); I <sup>2</sup> = 89%	6				
Test for overall effect: Z = 1.	18 (P = 0	.24)					decreased risk increased risk			

#### B Hypothyroidism

	Case		Control		Odds Ratio		Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	M-H, Fixed, 95% Cl
Abu-Hassan-hypo 2019	37	82	29	66	83.1%	1.05 [0.55, 2.01]	]
Kvaratskhelia-hypo 2020	6	32	4	26	16.9%	1.27 [0.32, 5.08]	]
Total (95% CI)		114		92	100.0%	1.09 [0.60, 1.96]	
Total events	43		33				
Heterogeneity: Chi <sup>2</sup> = 0.06,	df = 1 (P	= 0.81)	; l² = 0%				
Test for overall effect: Z = 0.27 (P = 0.78)							decreased risk increased risk

### 5.2 Figure of sensitivity analysis



5.3 Figure of trial sequence analysis (only Caucasians-subgroup analysis)

